FUNCTIONAL ASSESSMENT OF HYDROPHILIC DOMAINS OF LEA PROTEINS FROM DISTANT ORGANISMS

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Late embryogenesis abundant (LEA) proteins play a protective role during desiccation and oxidation stresses. LEA3 proteins are a major group characterized by a hydrophilic domain (HD) with a highly conserved repeating 11-amino acid motif. We compared four different HD orthologs from distant organisms: (i) DrHD from the extremophilic bacterium *Deinococcus radiodurans*; (ii) CeHD from the nematode *Caenorhabditis elegans*; (iii) YlHD from the yeast *Yarrowia lipolytica*; and (iv) BnHD from the plant *Brassica napus*. Circular dichroism spectroscopy showed that all four HDs were intrinsically disordered in phosphate buffer and then folded into a-helical structures with the addition of glycerol or trifluoroethanol. Heterologous HD expression conferred enhanced desiccation and oxidation tolerance to Escherichia coli. These four HDs protected the enzymatic activities of lactate dehydrogenase (LDH) by preventing its aggregation under desiccation stress. The HDs also interacted with LDH, which was intensified by the addition of hydrogen peroxide (H_2O_2), suggesting a protective role in a chaperone-like manner. Based on these results, the HDs of LEA3 proteins show promise as protectants for desiccation and oxidation stresses, especially DrHD, which is a potential ideal stress-response element that can be applied in synthetic biology due to its extraordinary protection and stress resistance ability.

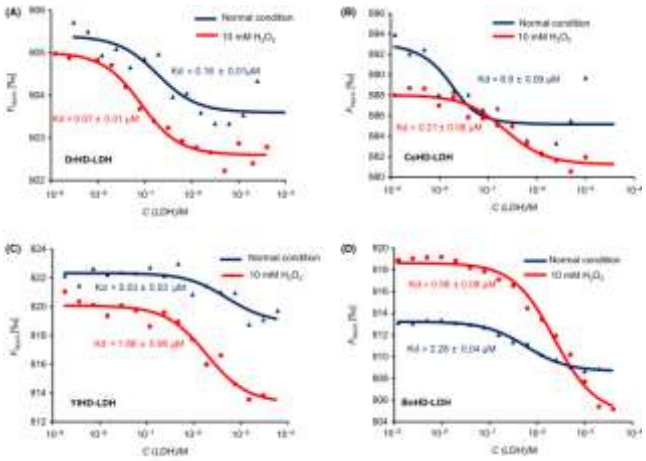

Figure1．Interaction of HDs with LDH.

A protein–protein interaction method, microscale electrophoresis, was employed to characterize the relationship between LDH and DrHD (A), CeHD (B), YlHD (C) and BnHD (D). The Kd value represents the dissociation constant indicating their affinity. The binding curve under normal conditions (phosphate buffer) is shown in dark blue, and the binding curve under 10 mM H_2O_2 treatment is shown in red